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ORIGINAL ARTICLE

Evaluation of Antacid Activity of *Garcinia Indica* Fruit Rind by a Modified Artificial Stomach Model

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ABSTRACT
The present study investigates the antacid effect of the aqueous extract of the fruit rind of Garcinia indica (GIE) by using a modified artificial stomach model. The pH of GIE (400 mg/kg and 800 mg/kg) and its neutralizing effect on artificial gastric acid was determined and compared with water and the active control sodium bicarbonate. A modified model of Vatier's artificial stomach was used to determine the duration of consistent neutralization of the artificial gastric acid. The neutralization capacity in vitro was determined with the classical titration method of Fordtran's model. All treatments including GIE 400 mg/kg, GIE 800 mg/kg and sodium bicarbonate showed significant acid neutralizing effects when compared with water. The duration for consistent neutralization and antacid capacities of GIE 400 mg/kg and 800 mg/kg were significantly higher than that of water. GIE (400 mg/kg and 800 mg/kg) was consistently active in the artificial stomach model and possesses potent antacid effects.
Key Words: *Garcinia indica fruit rind, antacid, modified artificial stomach, Fordtran's method*

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INTRODUCTION

Hyperacidity is a condition that occurs in many diseases caused by an excessive formation of acid in the stomach associated with a burning sensation in the throat and heart area. This condition appears in many diseases ranging from gastritis and peptic ulcers to the currently named gastro-esophageal reflux disease (GERD) [1].

Peptic ulcer or Peptic ulcer disease (PUD) is defined as a break in the mucosal lining of the gastrointestinal tract [2]. It occurs in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin, i.e. the stomach and duodenum. The normal stomach mucosa maintains a balance between defensive and aggressive factors [3]. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, free radicals, use of alcohol and nonsteroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (*Helicobacter pylori* and others). On the other hand defensive factors such as mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis, endogenous nitric oxide and normal tissue

microcirculation protect against ulcer formation. Although the etiology of ulcer is unknown yet, it is generally accepted that peptic ulcers develop when aggressive factors (endogenous, exogenous and/or infectious agents) overcome mucosal defense mechanisms [4]. The incidence of PUD varies with age, gender, geographical location and is associated with severe complications including haemorrhages, perforations, gastrointestinal obstruction, and malignancy. Thus, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss [5].

Modest approaches to control peptic ulceration include potentiation of the mucosal defense along with reduction of acid secretion and its neutralization, enhancement of antioxidant levels in the stomach, stimulation of gastric mucin synthesis and inhibition of *H. pylori* growth. The currently used drugs include antibiotics to kill *H. pylori*, acid blockers which reduce acid secretion for a prolonged duration (ranitidine, famotidine), proton pump inhibitors (omeprazole), and tissue lining protecting agents (sucralfate, bismuth) [6]. These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease, and are often expensive for the poor population.

In light of the above, it is pertinent to study natural products from food/plants as potential anti-ulcer compounds. Due to lesser side effects compared to synthetic drugs, currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care [7].

Garcinia indica (Family: Guttiferae; Clusiaceae), a slender evergreen tree is endemic to the west coast of India [8]. It has many culinary, pharmaceutical and industrial uses. The dried outer rind of fruits of *Garcinia indica* is popularly known as kokum and is used for imparting flavor and taste to curries. The fresh fruits are steeped in sugar syrup to make "amrut kokum" - a healthy soft drink to relive sunstroke and to provide gastric relief during summer. Kokum juice is mixed with yogurt and salt to make a "natural antacid" and has a plausible function as an anti-ulcer agent [9]. Many therapeutic effects of the fruit have been described in Ayurveda, which include its usefulness as an infusion, in skin ailments such as rashes caused by allergies; in treatment of burns, scalds and chaffed skin; as a remedy for dysentery and mucous diarrhoea; as an appetizer and a good liver tonic; as a cardiotonic and for bleeding, piles, dysentery, tumors and heart diseases [10]. The major phytoconstituents present in *G. indica* are anthocyanins, hydroxycitric acid (HCA), garcinol, isogarcinol and polyphenols. One phytoconstituent of kokum, hydroxycitric acid (HCA), has been patented for use as a hypocholesterolaemic agent [11]. Garcinol, a polyisoprenylated benzophenone purified from *G. indica* fruit rind displays anti-cancer and antioxidant activities [12]. Apart from HCA and garcinol, kokum contains other compounds like isogarcinol, ascorbic acid and polyphenols with potential antioxidant properties

Antacids cure ulcers through neutralization of gastric acid (HCl) and therefore are used in the treatment of PUD routinely. Keeping in view the use of kokum as an effective home remedy for acidity, the present study was undertaken to evaluate the antacid effect *Garcinia indica* fruit rind on gastric acid *in vitro* using the modified model of Vatier's artificial stomach and the titration method of Fordtran's model.

MATERIALS AND METHODS

Collection, authentication and extraction of fruit rinds

The fruit rind of *G. indica* was collected from the Konkan region of Maharashtra, India and air dried under shade, powdered mechanically and stored in air tight containers. The powder was extracted using soxhlet apparatus and water as a solvent and stored in a refrigerator for further use. The plant was authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai, India after matching with the existing specimen (accession no. 03587).

Chemicals and reagents

Pepsin and sodium chloride were purchased from Sigma Chemical Co., St Louis, MO, USA. Hydrochloric acid was obtained from Merck Ltd., Mumbai, India. Sodium bicarbonate was purchased from S.D. Fine Chemicals Ltd., Mumbai, India. All other chemicals were obtained from local sources and were of analytical grade.

Instruments

The instruments used in this experiment were a standard pH meter (LABINDIA, SAB 5000), a magnetic stirrer with hot plate temperature controller (1MLH, REMI), an adjustable electrode stand and a peristaltic tubing pump (ELECTROLAB PP 201 V).

Preparation of artificial gastric acid

Two grams of NaCl and 3.2 mg of pepsin were dissolved in 500 mL distilled water. Hydrochloric acid (7.0 mL) and adequate water were added to make a 1000 mL solution. The pH of the solution was adjusted to 1.20.

pH determination of the GIE

The pH of GIE (400 mg/kg and 800 mg/kg) was determined at temperatures ranging from 25^0C to 37^0C. The pH values of the active control solution sodium bicarbonate (SB) and water was also determined for comparison.

Determination of the neutralizing effects on artificial gastric acid

The freshly prepared test solutions GIE [400 mg/kg and 800 mg/kg (90 mL)], water (90 mL) and the active control SB (90 mL) were added separately to the artificial gastric juice (100 mL) at pH 1.2. The pH values were determined to examine the neutralizing effects on artificial gastric juice. (Six experiments were performed for each solution, Table 1)

Determination of the duration of consistent neutralization on artificial gastric acid using the modified model of Vatier's artificial stomach [13, 14]

The apparatus of the modified model of Vatier's artificial stomach was made up of three elements: a pH recording system (R), a stomach (S) and a peristaltic pump (P). The stomach was made up of three portions, S1, S2 and S3. S1 was a reservoir (container), S2 modeled the secretory flux (F-IN), and S3 modeled the gastric emptying flux (F-OUT). Each freshly prepared test sample (90 mL) was added to 100 mL of artificial gastric juice at pH 1.2 in the container of the artificial stomach at 37^0C and continuously

stirred (30 rpm) with a 2.5-cm magnetic stirring apparatus. Artificial gastric juice at pH 1.2 was pumped at 3 mL/min into the container of the artificial stomach, and pumped out at 3 mL/min at the same time. A pH meter was connected to continuously monitor the changes of pH in the container of the artificial stomach. The duration of the neutralization effect was determined when the pH value returned to its initial value (pH 1.2). Six experiments were performed for each freshly prepared test solution, water and standard (SB). (Table 2).

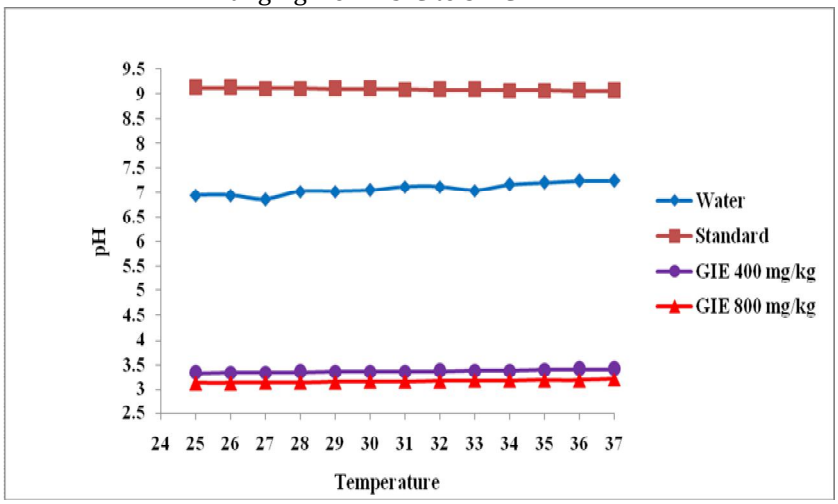
Determination of the neutralization capacity *in vitro* using the titration method of Fordtran's model [15, 16]

Each freshly prepared test sample (90 mL) was placed in a 250 mL beaker and warmed to 37^{0}C. A magnetic stirrer was continuously run at 30 rpm to imitate the stomach movements. The test samples were titrated with artificial gastric juice to the end point of pH 3. The consumed volume (V) of the artificial gastric juice was measured. The total consumed H^+ (mmol) was measured as 0.063096 (mmol/mL) × V (mL). Six experiments were performed for each freshly prepared test solution, water and standard (SB). (Table 3).

Statistical analysis

The results of antacid activity are expressed as mean ± SEM. Results were statistically analyzed using one-way ANOVA, followed by the Dunnet's post test for individual comparison. P<0.05 was considered to be significant.

Figure 1. pH values of water, standard and GIE (400mg/kg and 800mg/kg) determined at temperatures ranging from 25^{0}C to 37^{0}C.



GIE: Aqueous extract of *G. indica* fruit

Table 1. pH values with 90 ml water, standard and GIE (400mg/kg and 800mg/kg) added to 100 mL of artificial gastric juice

Drug	pH value
Water	1.52 ± 0.02
Standard	2.11 ± 0.14**
GIE 400 mg/kg	1.93 ± 0.08**
GIE 800 mg/kg	1.91 ± 0.02*

Data are presented as mean ± SEM (n = 6) *P < 0.05, **P < 0.01 when compared with water
GIE: Aqueous extract of *G. indica* fruit

Table 2. Duration of antacid effect for consistent neutralization of gastric acid

Drug	Time (min)
Water	108 ± 4.03
Standard	182 ± 7.67*
GIE 400 mg/kg	164 ± 4.31*
GIE 800 mg/kg	156 ± 10.56*

Data are presented as mean ± SEM (n = 6) *P < 0.01 when compared with water
GIE: Aqueous extract of *G. indica* fruit.

Table 3. Consumed volume of artificial gastric juice and H^+ (mmol) in the titration of 90 mL water, standard and GIE (400mg/kg and 800mg/kg) with artificial gastric juice (pH 1.2) to the end point of pH 3

Drug	Consumed volume of artificial gastric juice (mL)	mmol of H^+
Water	2.44 ± 0.11	0.15 ± 0.007
Standard	72.17 ± 1.20*	4.55 ± 0.08*
GIE 400 mg/kg	22.17 ± 0.75*	1.40 ± 0.05*
GIE 800 mg/kg	20.33 ± 0.95*	1.28 ± 0.06*

Data are presented as mean ± SEM (n = 6) *P < 0.01 when compard with water
GIE: Aqueous extract of *G. indica* fruit

RESULTS

pH values of the test solutions at temperatures ranging from 25^{0}C to 37^{0}C

The pH values of the GIE 400 mg/kg and GIE 800 mg/kg solutions at temperatures from 25^{0}C to 37^{0}C ranged from 3.33 to 3.41 and 3.12 to 3.20, respectively. The pH values of water and SB solutions at temperatures from 25^{0}C to 37^{0}C ranged from 6.94 to 7.24 and 9.13 to 9.07, respectively (Figure 1). The results indicate that temperature did not affect pH significantly.

Neutralizing effects on artificial gastric acids

When 90 mL of the test solution was added to 100 mL of the artificial gastric juice (pH 1.2), the pH values of GIE 400 mg/kg and GIE 800 mg/kg solutions were found to be 1.93 ± 0.08 and 1.91 ± 0.02, respectively. The pH values of water and SB solutions were 1.52 ± 0.02 and 2.11 ± 0.14, respectively. This result shows that the neutralizing effect of 400 mg/kg and GIE 800 mg/kg was significantly better than that of water (Table 1).

Duration of consistent neutralization effect on artificial gastric acids

The durations for consistent neutralizing effects of GIE 400 mg/kg and 800 mg/kg solutions were 164 ± 4 min and 156 ± 11 min, respectively. Those of water and SB solutions were 108 ± 4 and 182 ± 8 min, respectively. The duration of antacid action of SB was the longest, followed by the GIE 400 mg/kg and 800 mg/kg, which were significantly higher than that for water. (Table 2)

Physical neutralization capacity *in vitro*

The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, GIE 400 mg/kg, GIE 800 mg/kg and SB solutions were 2.44 ± 0.11, 22.17 ± 1.20, 20.33 ± 0.95 and 72.17 ± 1.20 mL, respectively. The consumed H^+ were 0.15 ± 0.007, 1.40 ± 0.08, 1.28 ± 0.06 and 4.55 ± 0.08 mmol, respectively (Table 3). The active control SB and both tests (GIE 400 mg/kg and 800 mg/kg), exhibited significant antacid potency. The neutralization capacities of GIE 400 mg/kg and 800 mg/kg were lesser than that of SB but significantly better than that of water.

DISCUSSION

There have recently been surprising advances in the understanding of the pathophysiology and treatment of peptic ulcer disease (PUD).. The ability of the gastric mucosa to resist injuries by endogenous secretions (acid, pepsin and bile), and by ingested irritants (e.g. alcohol and NSAIDs) is attributed to a number of factors that have been collectively referred to as “mucosal defence” [3,4]. Damage of this “mucosal defence” is an initial step in ulcer development and has been known to be primarily due to hypersecretion of HCl and oxidative stress by Reactive Oxygen Species (ROS) [3,5]. The principal treatment of PUD includes antacids, H2 receptor antagonists and proton pump inhibitors. Among these, antacids have been widely used in the treatment of ulcers for many years. Antacids act by neutralizing gastric acid and help in healing of ulcers, though, they do not decrease the volume of gastric secretion. Antacids are prescribed on the basis of their *in vivo* and *in vitro* potency [17]. Potency, cost, taste, salt content, bowel habit, and side effects are important factors considered in the choice of antacids [17]. Side effects and drug interactions are major clinical problems associated with antacid therapy. Therefore, traditional herbal medicines have recently generated increasing interest for the treatment of ulcer disease [18].

The *G. indica* fruit (kokum) juice is traditionally used for the treatment of acidity. Therefore, the present study used the titration method of Fordtran’s model and the modified model of Vatier’s artificial stomach, which mimic the regular physiological functioning of a human stomach, to explore the antacid effects of the *G. indica* fruit rind. The model of the 'artificial stomach' reproduces two major gastric dynamic functions. It allows interplay between one function (F-IN) corresponding to acid secretion and the other (F-OUT) corresponding to gastric emptying. This model is used to mimic gastric secretion and emptying in physiological situations. A 3 ml/min rate of 0.1 N HCl introduction corresponds to an hourly acid output of 18 mmol whilst arbitrarily chosen emptying rates represent slow, equal to input and fast

emptyings. These parallel conditions are encountered in response to liquid meals in normal subjects. The lag-times before reaching selected pH values after introduction of the test solutions have been converted into antacid capacities (mmol H+), taking into account the amount of acid present in the 'stomach' at time 0 and the amount of acid introduced thereafter [13].

Efficacious, intensive antacid therapy is often unacceptable because of the common side effects, especially altered bowel functions. Aluminum salts may cause constipation and magnesium salts cause diarrhoea. Sodium bicarbonate (SB) should be avoided even though it is a potent neutralizer of acid because it contains significant amounts of sodium and may alter the systemic pH. In addition, antacid-drug interactions have been frequently reported for SB [19]. The most clinically significant interactions occur with ferrous sulfate, tetracycline and quinolone antibiotics. Other interactions are potentially significant because they involve drugs with narrow therapeutic ranges.

Considering the side effects and interactions of antacids, the natural and edible products such as *G. indica* fruits should be looked to as an alternative for the treatment of PUD. The present study, thus, aims to validate the traditional antacid claims of *Garcinia indica* fruit rind (kokum) and promote its use in the form of a soft drink and as a culinary spice in people's diets. Besides, it is cheap, readily available to all strata of society, with medicinal properties attributed to it.

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